

embodiment of the invention is a well expressed recombinant *Plasmodium* CelTOS protein utilizing codon harmonization. The technique for codon harmonization is disclosed in Published U.S. application Ser. No. 10/677,641 to Kincaid et al. which is incorporated by referenced in its entirety herein.

[0013] Another embodiment of the invention is a recombinant subunit CelTOS malaria vaccine expressed in *E. coli* utilizing a soluble, high yielding purification process.

[0014] Yet another embodiment of the invention is a method of inducing antibodies induced against CelTOS that are reactive against malarial sporozoites to prevent liver stage infection.

[0015] A alternate embodiment of the invention is a method of inducing antibodies induced against CelTOS that are reactive against malarial ookinets in the mosquito midgut preventing development of infectious salivary gland sporozoites

[0016] Another embodiment of the invention is a pre-erythrocytic stage malaria vaccine to be used alone or in combination with alternate treatment strategies.

[0017] A further embodiment of the invention is as a pre-erythrocytic biomarker for exposure to malaria.

[0018] Another embodiment of the invention to use CelTOS antigens as a reagent for stimulating cells, T cells and B cells.

[0019] It is yet another embodiment of the invention to use CelTOS antigens as a reagent for evaluating antibody responses by ELISA, Luminex, or similar technologies well known in the art.

[0020] An additional embodiment of the invention is as a reagent used to develop monoclonal antibodies against the target *Plasmodium* parasite. Such monoclonal antibodies can potentially be used for passive immunotherapy.

[0021] Another embodiment of the invention is as a reagent to study protein folding and structure.

[0022] The various features of novelty that characterize the invention are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its operating advantages and specific objects attained by its uses, reference is made to the accompanying drawings and descriptive matter in which a preferred embodiment of the invention is illustrated.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] In the drawings:

[0024] FIG. 1 is a table showing *P. berghei* challenge and protection results in BalbC Mice. BalbC mice were immunized subcutaneously in the inguinal area at 3 week intervals with either a low (1 µg) dose or high dose (10 µg) of recombinant protein in a vaccine adjuvant such as MONTANIDE TM ISA 720 (A.L.A. Intermountain, 3725 E. Washington Street, Phoenix, Ariz. 85034). Mice were challenged with a total of 4000 *P. berghei* sporozoites, subcutaneously at 2 sites in the inguinal area. Blood smears were made on Day 6, Day 8, Day 10, Day 12 and Day 14 post challenge to determine infectivity. Results are shown as number of mice infected out of 5 mice per group.

[0025] FIG. 2 is a table showing *P. berghei* challenge and protection results in ICR mice immunized subcutaneously with a recombinant protein and a vaccine adjuvant. ICR mice were immunized subcutaneously, inguinal area, 3 week intervals with a low (1 µg) dose or high dose (10 µg) of recombinant protein and a vaccine adjuvant such as MONTANIDE TM ISA 720 (A.L.A. Intermountain, 3725E. Washington Street, Phoenix, Ariz. 85034). Mice were challenged with *P.*

berghei sporozoites, subcutaneously at 2 sites in the inguinal area. Blood smears were made on Day 6, Day 8, Day 10, Day 12 and Day 14 post challenge to determine infectivity. Results are Number of Mice that became infected over the total.

[0026] FIG. 3 is a table showing *P. berghei* challenge and protection results in BalbC mice immunized subcutaneously with a recombinant protein and a vaccine adjuvant. BalbC mice were immunized subcutaneously, inguinal area, 3 week intervals with a low (1 µg) dose or high dose (10 µg) of recombinant protein and a vaccine adjuvant such as MONTANIDE TM ISA 720 (A.L.A. Intermountain, 3725 E. Washington Street, Phoenix, Ariz. 85034). Mice were challenged with *P. berghei* sporozoites, subcutaneously at 2 sites in the inguinal area. Blood smears were made on Day 6, Day 8, Day 10, Day 12 and Day 14 post challenge to determine infectivity. Results are Number of Mice that became infected over the total.

[0027] FIG. 4 is a *Plasmodium falciparum* CelTOS nucleotide sequence which was optimized by codon harmonization for expression (SEQ. ID. NO. 1)

[0028] FIG. 5 is a *Plasmodium falciparum* CelTOS peptide sequence (SEQ. ID. NO. 2)

[0029] FIG. 6 is a translation map for the 528 residue sequence of *Plasmodium falciparum* CelTOS (PfCelTOS).

[0030] FIG. 7 is a diagram showing the construction of the pET(K-)-PfCelTOS plasmid.

[0031] FIG. 8 is a diagram showing the construction of the pET(K-)-PbCelTOS plasmid.

[0032] FIG. 9 is a diagram showing the construction of the pCI-TPA-PfCelTOS plasmid.

[0033] FIG. 10 is a diagram showing the construction of the pCI-TPA-PbCelTOS plasmid.

[0034] FIG. 11A is a photograph of an electrophoresis gel panel showing recombinant PbCelTOS and PfCelTOS expression profiles in *E. Coli* Lysates.

[0035] FIG. 11B is a photograph of an electrophoresis gel panel showing recombinant PbCelTOS and PfCelTOS expression profiles in *E. Coli* Lysates probed with mAb against His-tag.

[0036] FIG. 12 is a sequence generated by the NetNGlyc 1.0 Server at the Technical University of Denmark. The NetNGlyc server predicts N-Glycosylation sites in human proteins using artificial neural networks that examine the sequence context of Asn-Xaa-Ser/Thr sequons. See Prediction of N-glycosylation sites in human proteins. R. Gupta, E. Jung and S. Brunak. In preparation, 2004 incorporated herein by reference in its entirety. Asn-Xaa-Ser/Thr sequons in the sequence are in bold. Asparagines predicted to be N-glycosylated are underlined. Proteins without signal peptides are unlikely to be exposed to the N-glycosylation machinery and thus may not be glycosylated (in vivo) even though they contain potential motifs. (SEQ. ID. NO. 3).

[0037] FIG. 13 is a summary ELISA Antibody from pooled groups of Study No. 1.

[0038] FIG. 14A is a photograph of an electrophoresis gel showing high soluble protein yields of the PbCelTOS construct.

[0039] FIG. 14B is a photograph of an electrophoresis gel showing high soluble protein yields of the PfCelTOS construct.

[0040] FIG. 15A is photograph of *Plasmodium falciparum* sporozoites pre-incubated with control serum and immunofluorescent treatment.